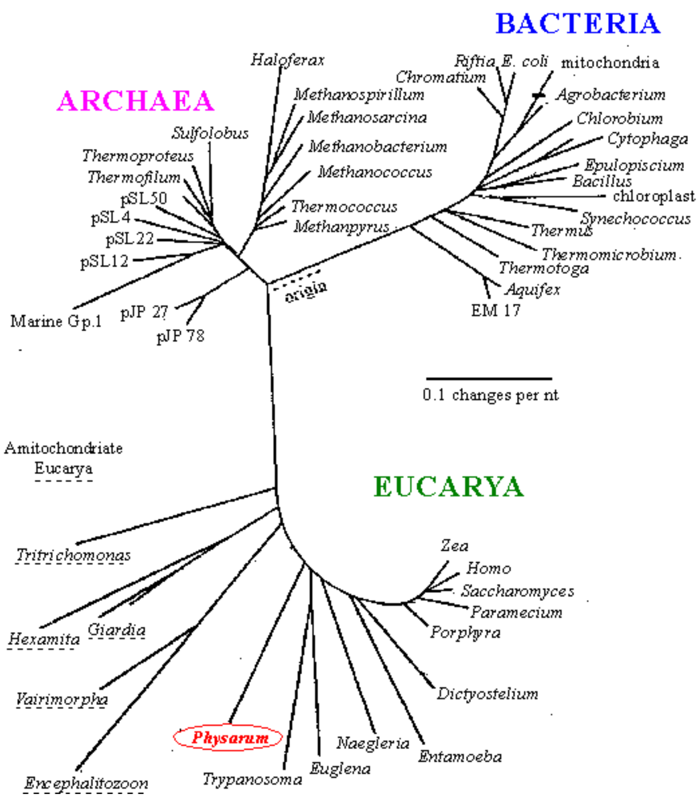
Pairwise Alignment

Anders Gorm Pedersen
Molecular Evolution Group
Center for Biological Sequence Analysis

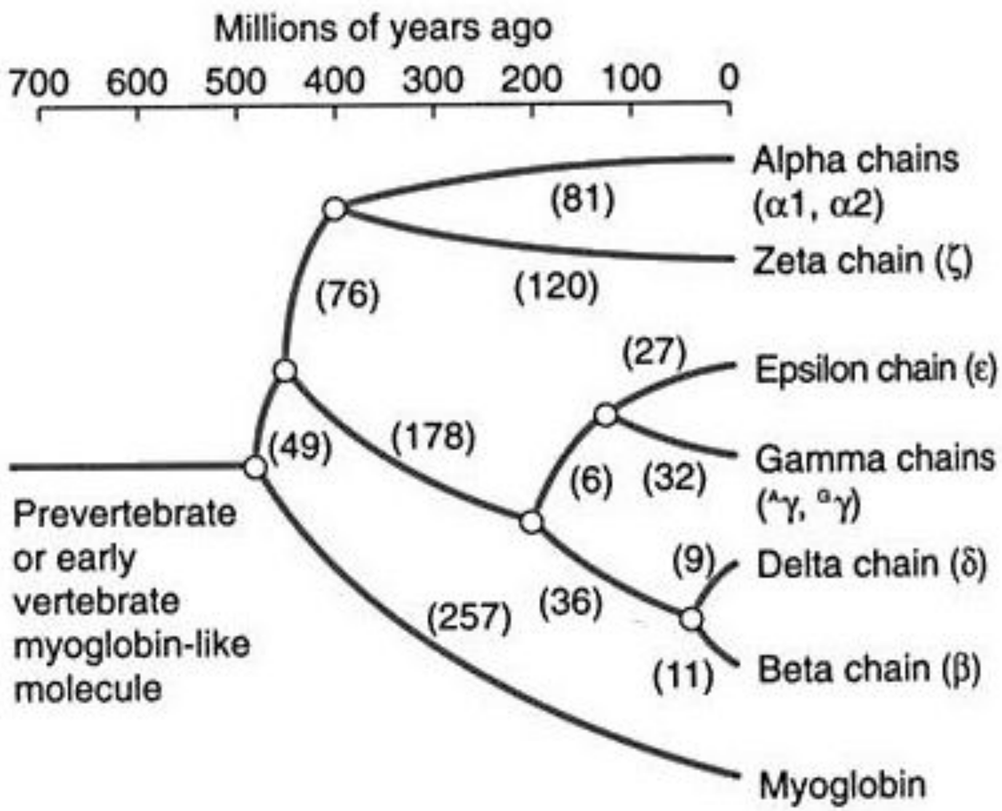
Sequences are related

• Darwin: all organisms are related through descent with modification
• => Sequences are related through descent with modification
• => Similar molecules have similar functions in different organisms



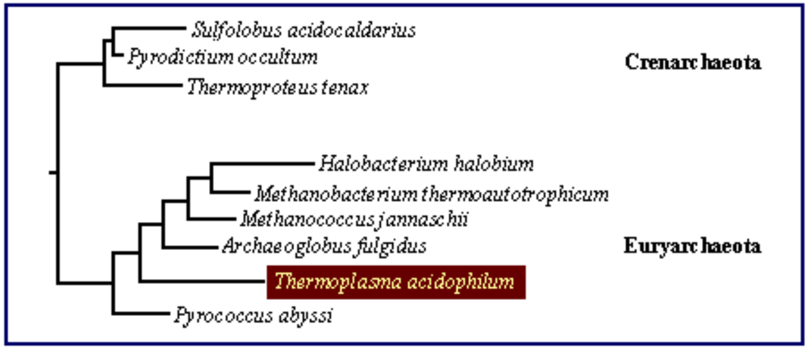
Phylogenetic tree based on ribosomal RNA:
three domains of life

Sequences are related, II



Phylogenetic tree of globin-type proteins found in humans

Why compare sequences?



- Determination of evolutionary relationships

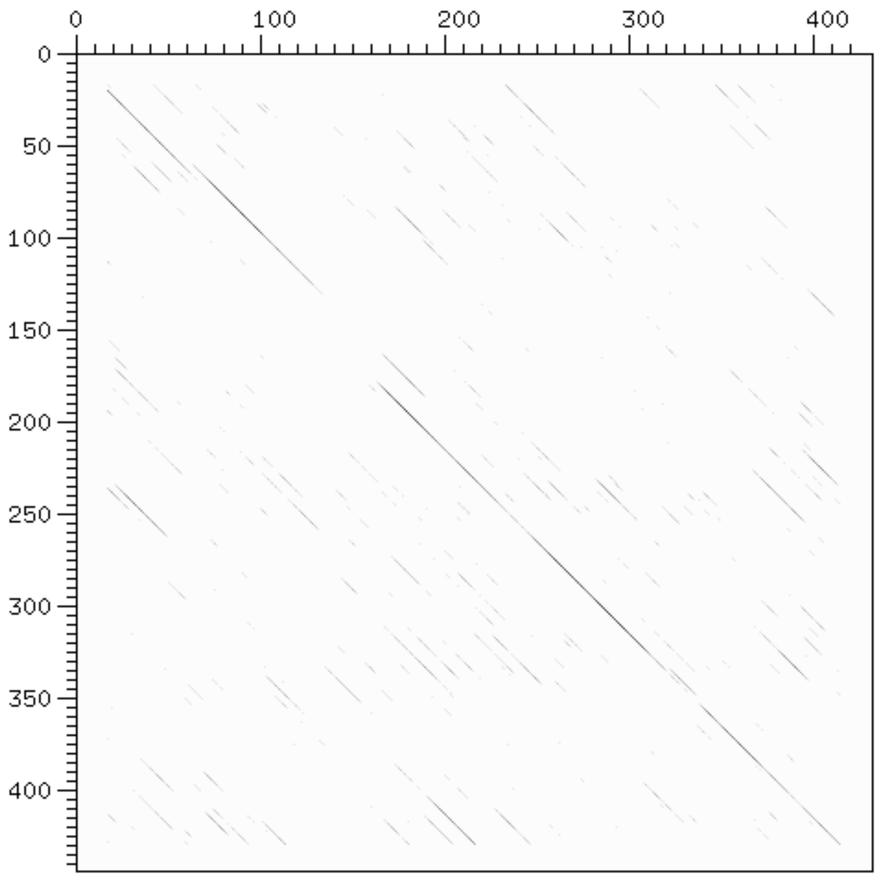
Protein 1: binds oxygen

Sequence similarity

Protein 2: binds oxygen ?

- Prediction of protein function and structure (database searches).

Dotplots: visual sequence comparison



1. Place two sequences along axes of plot

2. Place dot at grid points where two sequences have identical residues

3. Diagonals correspond to conserved regions

Pairwise alignments

```
43.2% identity;                  Global alignment score: 374

                10        20        30        40              50
alpha  V-LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS-----HGSA
       : :.: .:. : : ::::  .. : :.::: :... .: :. .:  : :::      :.
beta   VHLTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNP
               10          20        30        40        50

            60        70        80        90       100       110
alpha  QVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHL
       .::.:::::  :.....::.:.. .....::.::  ::.::: ::.::.. :. .:: :.
beta   KVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHF
       60        70        80        90       100       110

           120       130       140
alpha  PAEFTPAVHASLDKFLASVSTVLTSKYR
         :::: :.:. .: .:.:...:. ::.
beta   GKEFTPPVQAAYQKVVAGVANALAHKYH
      120       130       140
```

Pairwise alignment

```
100.000% identity in 3 aa overlap

              SPA
              :::
              SPA
```

Percent identity is not a good measure of alignment quality

Pairwise alignments: alignment score

```
43.2% identity;                   Global alignment score: 374

                10        20        30        40              50
alpha  V-LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS-----HGSA
       : :.: .:. : : ::::  .. : :.::: :... .: :. .:  : :::      :.
beta   VHLTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNP
               10          20        30        40        50

            60        70        80        90       100       110
alpha  QVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHL
       .::.:::::  :.....::.:.. .....::.::  ::.::: ::.::.. :. .:: :.
beta   KVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHF
       60        70        80        90       100       110

           120       130       140
alpha  PAEFTPAVHASLDKFLASVSTVLTSKYR
         :::: :.:. .: .:.:...:. ::.
beta   GKEFTPPVQAAYQKVVAGVANALAHKYH
      120       130       140
```

Alignment scores: match vs. mismatch

Simple scoring scheme (too simple in fact…):

Matching amino acids: 5
Mismatch: 0

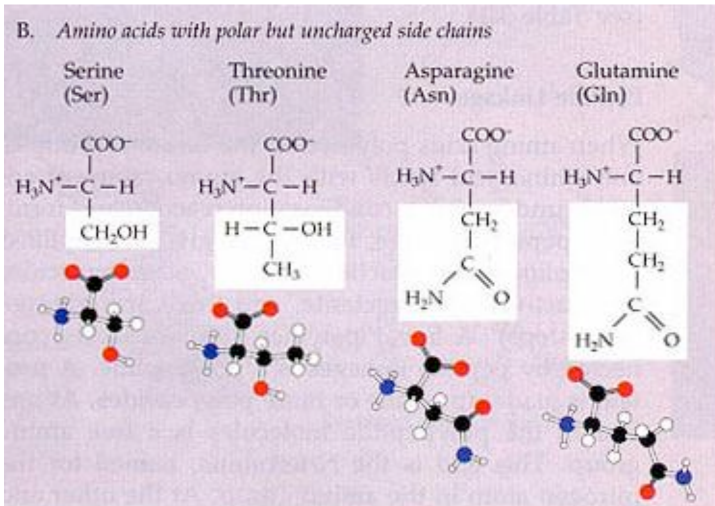
Scoring example:

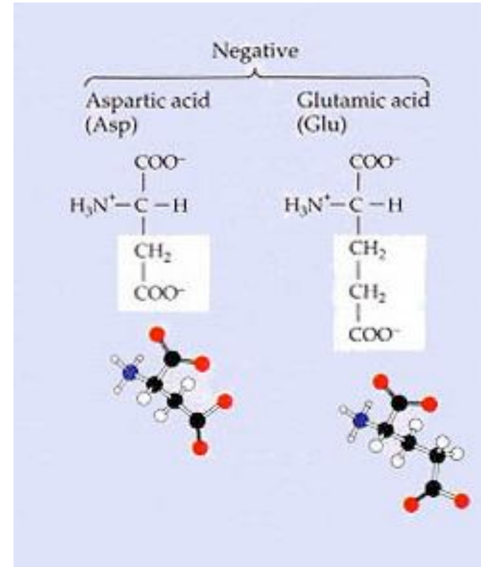
```
K A W S A D V
:   : : :   :
K D W S A E V
5+0+5+5+5+0+5  =  25
```

Pairwise alignments: conservative substitutions

```
43.2% identity;                  Global alignment score: 374

                10        20        30        40              50
alpha  V-LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS-----HGSA
       : :.: .:. : : ::::  .. : :.::: :... .: :. .:  : :::      :.
beta   VHLTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNP
               10          20        30        40        50

            60        70        80        90       100       110
alpha  QVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHL
       .::.:::::  :.....::.:.. .....::.::  ::.::: ::.::.. :. .:: :.
beta   KVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHF
       60        70        80        90       100       110

           120       130       140
alpha  PAEFTPAVHASLDKFLASVSTVLTSKYR
         :::: :.:. .: .:.:...:. ::.
beta   GKEFTPPVQAAYQKVVAGVANALAHKYH
      120       130       140
```

Amino acid properties





Serine (S) and Threonine (T) have similar physicochemical properties

Aspartic acid (D) and Glutamic acid (E) have similar properties

=> Substitution of S/T or E/D occurs relatively often during evolution

=> Substitution of S/T or E/D should result in scores that are only moderately lower than identities

Protein substitution matrices

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5																			
R	−2	7																		
N	−1	−1	7																	
D	−2	−2	**2**	**8**																
C	−1	−4	−2	−4	13															
Q	−1	1	0	0	−3	7														
E	−1	0	0	**2**	−3	**2**	**6**													
G	0	−3	0	−1	−3	−2	−3	8												
H	−2	0	**1**	−1	−3	**1**	0	−2	10											
I	−1	−4	−3	−4	−2	−3	−4	−4	−4	5										
L	−2	−3	−4	−4	−2	−2	−3	−4	−3	**2**	5									
K	−1	**3**	0	−1	−3	**2**	**1**	−2	0	−3	−3	6								
M	−1	−2	−2	−4	−2	0	−2	−3	−1	**2**	**3**	−2	7							
F	−3	−3	−4	−5	−2	−4	−3	−4	−1	0	**1**	−4	0	8						
P	−1	−3	−2	−1	−4	−1	−1	−2	−2	−3	−4	−1	−3	−4	10					
S	**1**	−1	**1**	0	−1	0	−1	0	−1	−3	−3	0	−2	−3	−1	**5**				
T	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	**2**	**5**			
W	−3	−3	−4	−5	−5	−1	−3	−3	−3	−3	−2	−3	−1	**1**	−4	−4	−3	15		
Y	−2	−1	−2	−3	−3	−1	−2	−3	**2**	−1	−1	−2	0	**4**	−3	−2	−2	**2**	8	
V	0	−3	−3	−4	−1	−3	−3	−4	−4	**4**	**1**	−3	**1**	−1	−3	−2	0	−3	−1	5

BLOSUM50 matrix:

- Positive scores on diagonal (identities)
- Similar residues get higher (positive) scores
- Dissimilar residues get smaller (negative) scores

Pairwise alignments: insertions/deletions

```
43.2% identity;                   Global alignment score: 374

                10        20        30        40              50
alpha  V-LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS-----HGSA
       : :.: .:. : : ::::  .. : :.::: :... .: :. .:  : :::      :.
beta   VHLTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNP
               10          20        30        40        50

            60        70        80        90       100       110
alpha  QVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHL
       .::.:::::  :.....::.:.. .....::.::  ::.::: ::.::.. :. .:: :.
beta   KVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHF
       60        70        80        90       100       110

           120       130       140
alpha  PAEFTPAVHASLDKFLASVSTVLTSKYR
         :::: :.:. .: .:.:...:. ::.
beta   GKEFTPPVQAAYQKVVAGVANALAHKYH
      120       130       140
```

Alignment scores: insertions/deletions

```
K L A A S V I L S D A L
K L A A - - - - S D A L
```

-10 + 3 x (-1)=-13

Affine gap penalties:

Multiple insertions/deletions may be one evolutionary event =>

Separate penalties for gap opening and gap elongation

Handout

Compute 4 alignment scores: two different alignments using two different alignment matrices (and the same gap penalty system)

Score 1: Alignment 1 + BLOSUM-50 matrix + gaps

Score 2: Alignment 1 + BLOSUM-Trp matrix + gaps

Score 3: Alignment 2 + BLOSUM-50 matrix + gaps

Score 4: Alignment 2 + BLOSUM-Trp matrix + gaps

Note: fake matrix constructed for pedagogic purposes.

Handout: summary of results

	Alignment 1	Alignment 2
BLOSUM-50	38	51
BLOSUM-Trp	118	91

Protein substitution matrices

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5																			
R	-2	7																		
N	-1	-1	7																	
D	-2	-2	2	8																
C	-1	-4	-2	-4	13															
Q	-1	1	0	0	-3	7														
E	-1	0	0	2	-3	2	6													
G	0	-3	0	-1	-3	-2	-3	8												
H	-2	0	1	-1	-3	1	0	-2	10											
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5										
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5									
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6								
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7							
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8						
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10					
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	2	5			
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15		
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

BLOSUM50 matrix:

- Positive scores on diagonal (identities)
- Similar residues get higher (positive) scores
- Dissimilar residues get smaller (negative) scores

Protein substitution matrices: different types

- **Identity matrix**
  (match vs. mismatch)

- **Chemical properties matrix**
  (use knowledge of physicochemical properties to design matrix)

- **Empirical matrices**
  (based on observed pair-frequencies in hand-made alignments)
  - PAM series
  - BLOSUM series
  - Gonnet

Estimation of the BLOSUM 62 matrix

- BLOSUM matrices are computed based on gap-free alignments in the so-called BLOCKS database. BLOSUM 62 is computed by comparing sequences that are less than 62% identical. BLOSUM 80 is computed from sequences less than 80% identical, etc.

- All pairs of sequences in a block are compared, and the **observed pair frequencies** (p_{ab}) are noted. For instance: p_{WW} = 0.0065, p_{AL} = 0.0044, etc.

- **Expected pair frequencies** are computed from single amino acid frequencies. For instance: $p_A \times p_L$ = 0.074 x 0.099 = 0.0073

- For each amino acid pair the substitution scores are essentially computed as follows (here λ is a scaling factor, used to obtain integer scores):

$$S(a,b) = \frac{1}{\lambda} \ln\left(\frac{p_{\text{obs}}}{p_{\text{exp}}}\right) = \frac{1}{\lambda} \ln\left(\frac{p_{ab}}{p_a \times p_b}\right)$$

```
ID   FIBRONECTIN_2; BLOCK
COG9_CANFA  GNSAGEPCVFPFIFLGKQYSTCTREGRGDGHLWCATT
COG9_RABIT  GNADGAPCHFPFTFEGRSYTACTTDGRSDGMAWCSTT
FA12_HUMAN  LTVTGEPCHFPFQYHRQLYHKCTHKGRPGPQPWCATT
HGFA_HUMAN  LTEDGRPCRFPFRYGGRMLHACTSEGSAHRKWCATTH
MANR_HUMAN  GNANGATCAFPFKFENKWYADCTSAGRSDGWLWCGTT
MPRI_MOUSE  ETDDGEPCVFPFIYKGKSYDECVLEGRAKLWCSKTAN
PB1_PIG     AITSDDKCVFPFIYKGNLYFDCTLHDSTYYWCSVTTY
SFP1_BOVIN  ELPEDEECVFPFVYRNRKHFDCTVHGSLFPWCSLDAD
SFP3_BOVIN  AETKDNKCVFPFIYGNKKYFDCTLHGSLFLWCSLDAD
SFP4_BOVIN  AVFEGPACAFPFTYKGKKYYMCTRKNSVLLWCSLDTE
SP1_HORSE   AATDYAKCAFPFVYRGQTYDRCTTDGSLFRISWCSVT
COG2_CHICK  GNSEGAPCVFPFIFLGNKYDSCTSAGRNDGKLWCAST
COG2_HUMAN  GNSEGAPCVFPFTFLGNKYESCTSAGRSDGKMWCATT
COG2_MOUSE  GNSEGAPCVFPFTFLGNKYESCTSAGRNDGKVWCATT
COG2_RABIT  GNSEGAPCVFPFTFLGNKYESCTSAGRSDGKMWCATS
COG2_RAT    GNSEGAPCVFPFTFLGNKYESCTSAGRNDGKVWCATT
COG9_BOVIN  GNADGKPCVFPFTFQGRTYSACTSDGRSDGYRWCATT
COG9_HUMAN  GNADGKPCQFPFIFQGQSYSACTTDGRSDGYRWCATT
COG9_MOUSE  GNGEGKPCVFPFIFEGRSYSACTTKGRSDGYRWCATT
COG9_RAT    GNGDGKPCVFPFIFEGHSYSACTTKGRSDGYRWCATT
FINC_BOVIN  GNSNGALCHFPFLYNNHNYTDCTSEGRRDNMKWCGTT
FINC_HUMAN  GNSNGALCHFPFLYNNHNYTDCTSEGRRDNMKWCGTT
FINC_RAT    GNSNGALCHFPFLYSNRNYSDCTSEGRRDNMKWCGTT
MPRI_BOVIN  ETEDGEPCVFPFVFNGKSYEECVVESRARLWCATTAN
MPRI_HUMAN  ETDDGVPCVFPFIFNGKSYEECIIESRAKLWCSTTAD
PA2R_BOVIN  GNAHGTPCMFPFQYNQQWHHECTREGREDNLLWCATT
PA2R_RABIT  GNAHGTPCMFPFQYNHQWHHECTREGRQDDSLWCATT
```

Estimation of the BLOSUM 62 matrix

- Example: A + L:

$$
\begin{aligned}
p_A &= 0.074, p_L = 0.099 \Rightarrow p_A \times p_L = 0.0073 \\
p_{AL} &= 0.044 \\
S_{AL} &= \frac{1}{\lambda} \ln\left(\frac{p_{AL}}{p_A \times p_L}\right) \\
&= \frac{1}{0.347} \ln\left(\frac{0.0044}{0.0073}\right) \\
&= \frac{-0.51}{0.347} \\
&= -1.46 \\
&\approx -1
\end{aligned}
$$

Pairwise alignment

Optimal alignment:

alignment having the highest possible score given a substitution matrix and a set of gap penalties

So:

best alignment can be found by exhaustively searching all possible alignments, scoring each of them and choosing the one with the highest score?

The problem: How many possible alignments are there?

```
ACG     AC-G    --ACG   -A-CG
ACG     ACG-    AC-G-   A-CG-

-ACG    AC-G    --ACG   ...
ACG-    A-CG    A-CG-

-ACG    AC-G    --ACG
AC-G    -ACG    AC--G

-ACG    ACG-    --ACG
A-CG    AC-G    A-C-G

A-CG    ACG-    --ACG
ACG-    A-CG    A--CG

A-CG    ACG-    -A-CG
AC-G    -ACG    ACG--

A-CG    --ACG   -A-CG
-ACG    ACG--   AC-G-
```

How many possible alignments are there?

Length of sequences: $n_1 = n_2$	Number of possible alignments
2	13
3	63
4	321
5	1683
10	8,097,453
20	2.61×10^{14}
100	2.05×10^{75}
300	1.53×10^{228}

Pairwise alignment: the problem

The number of possible pairwise alignments increases explosively with the length of the sequences:

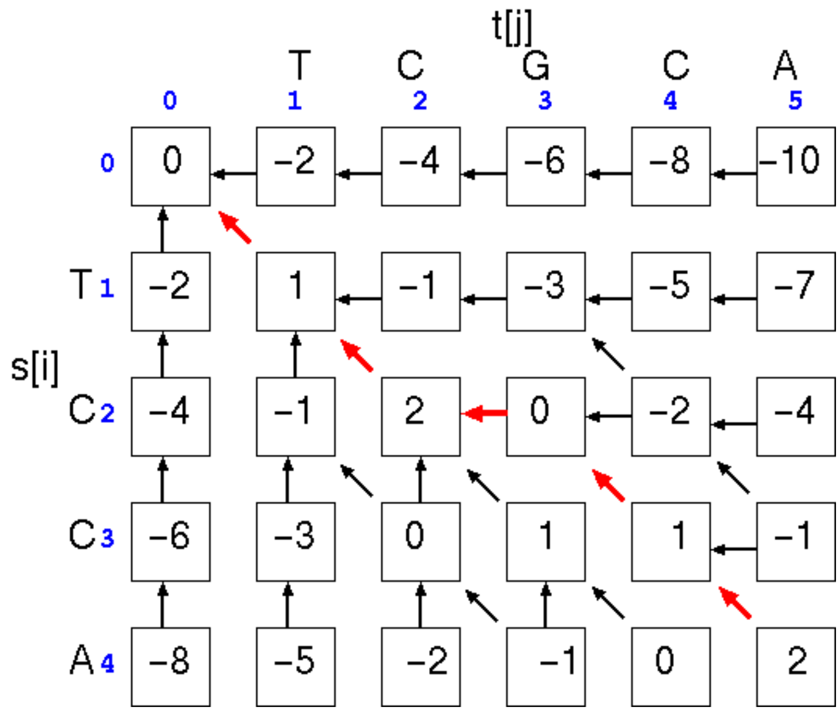
$$N = \sum_{k=0}^{n_2} \binom{n_1 + k}{n_2} \binom{n_2}{k}$$

Two protein sequences of length 300 amino acids can be aligned in approximately 10^{228} different ways

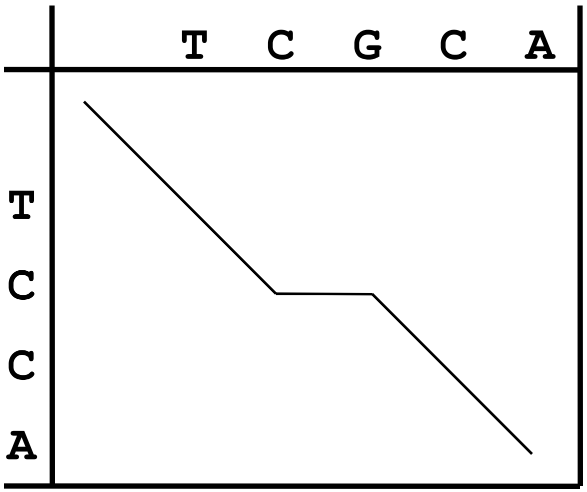
Time needed to test all possibilities much larger than the entire lifetime of the universe.

Pairwise alignment: the solution

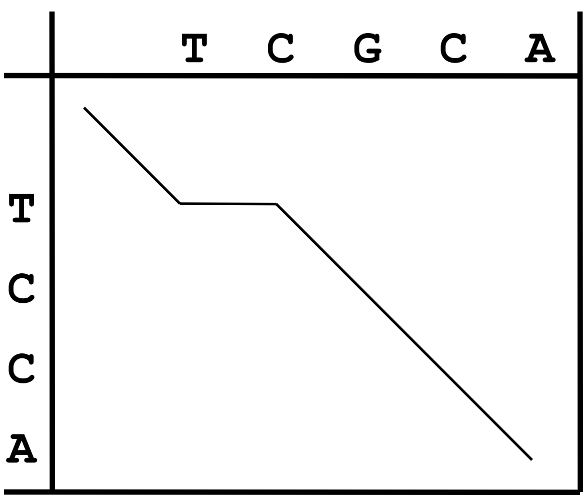
"Dynamic programming"
(the Needleman-Wunsch algorithm)



Alignment depicted as path in matrix

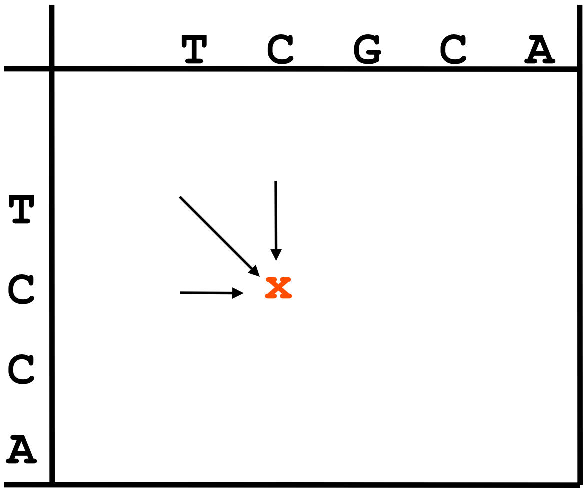



```
TCGCA
TC-CA
```




```
TCGCA
T-CCA
```

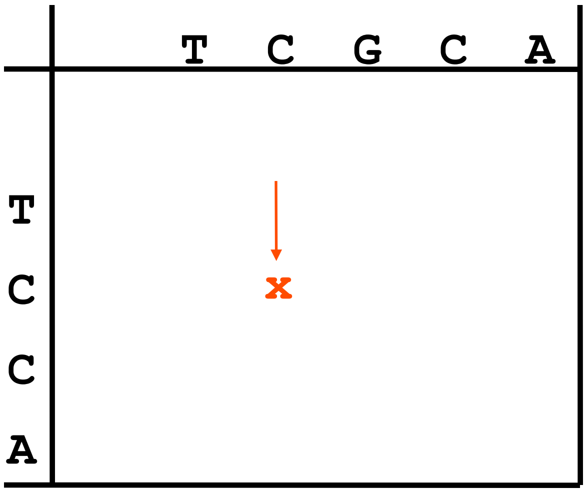
Dynamic programming: computation of scores



Any given point in matrix can only be reached from three possible previous positions (you cannot “align backwards”).

=> Best scoring alignment ending in any given point in the matrix can be found by choosing the highest scoring of the three possibilities.

Dynamic programming: computation of scores

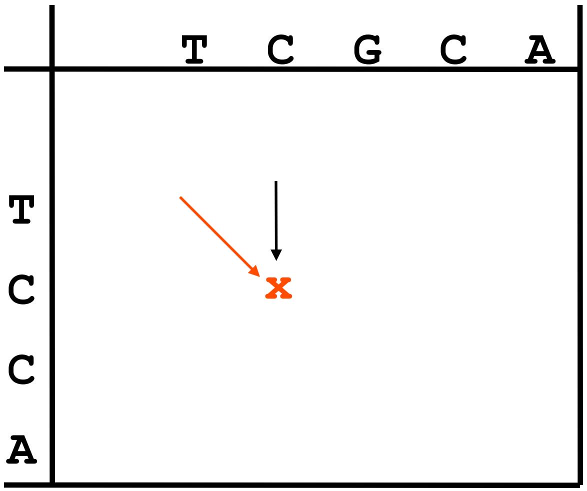


Any given point in matrix can only be reached from three possible positions (you cannot “align backwards”).

=> Best scoring alignment ending in any given point in the matrix can be found by choosing the highest scoring of the three possibilities.

$$\text{score}(x,y) = \max \begin{cases} \text{score}(x,y-1) - \text{gap-penalty} \\ \\ \end{cases}$$

Dynamic programming: computation of scores

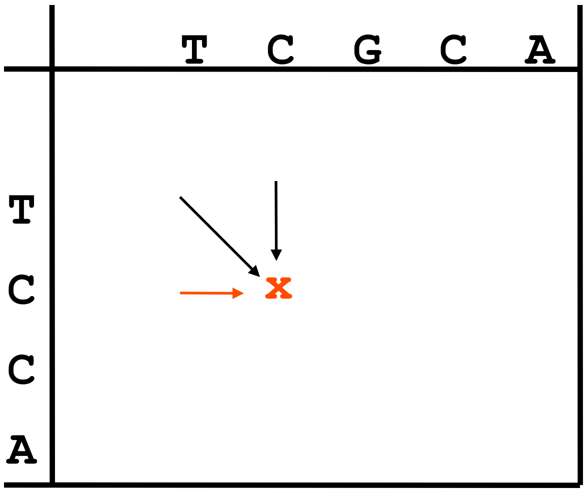


Any given point in matrix can only be reached from three possible positions (you cannot "align backwards").

=> Best scoring alignment ending in any given point in the matrix can be found by choosing the highest scoring of the three possibilities.

score(x,y) = max {
score(x,y-1) - gap-penalty
score(x-1,y-1) + substitution-score(x,y)
}

Dynamic programming: computation of scores

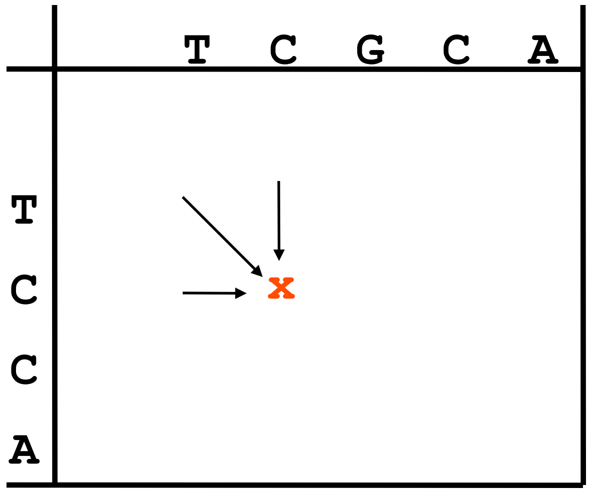


Any given point in matrix can only be reached from three possible positions (you cannot “align backwards”).

=> Best scoring alignment ending in any given point in the matrix can be found by choosing the highest scoring of the three possibilities.

$$\text{score}(x,y) = \max \begin{cases} \text{score}(x,y-1) - \text{gap-penalty} \\ \text{score}(x-1,y-1) + \text{substitution-score}(x,y) \\ \text{score}(x-1,y) - \text{gap-penalty} \end{cases}$$

Dynamic programming: computation of scores



Any given point in matrix can only be reached from three possible positions (you cannot "align backwards").

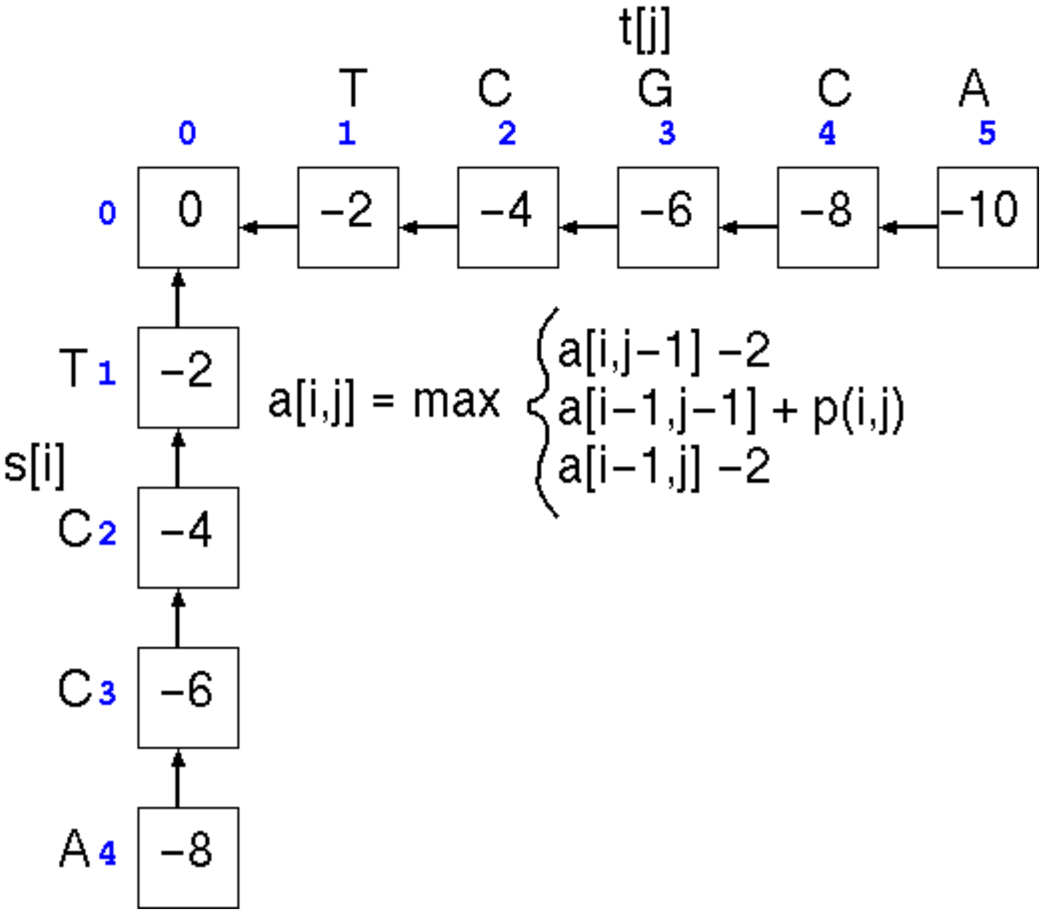
=> Best scoring alignment ending in any given point in the matrix can be found by choosing the highest scoring of the three possibilities.

Each new score is found by choosing the maximum of three possibilities. For each square in matrix: keep track of where best score came from.

Fill in scores one row at a time, starting in upper left corner of matrix, ending in lower right corner.

$$\text{score}(x,y) = \max \begin{cases} \text{score}(x,y-1) - \text{gap-penalty} \\ \text{score}(x-1,y-1) + \text{substitution-score}(x,y) \\ \text{score}(x-1,y) - \text{gap-penalty} \end{cases}$$

Dynamic programming: example

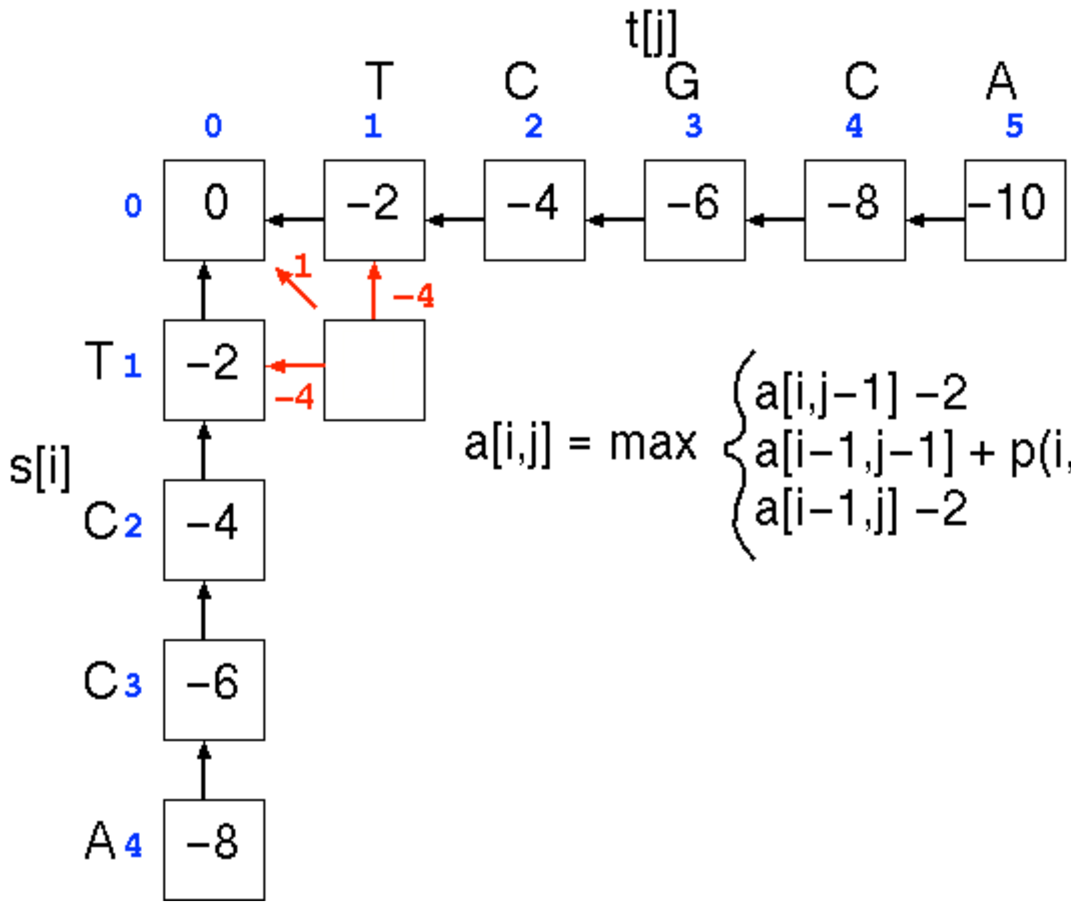


$$a[i,j] = \max \begin{cases} a[i,j-1] - 2 \\ a[i-1,j-1] + p(i,j) \\ a[i-1,j] - 2 \end{cases}$$

	A	C	G	T
A	1	-1	-1	-1
C	-1	1	-1	-1
G	-1	-1	1	-1
T	-1	-1	-1	1

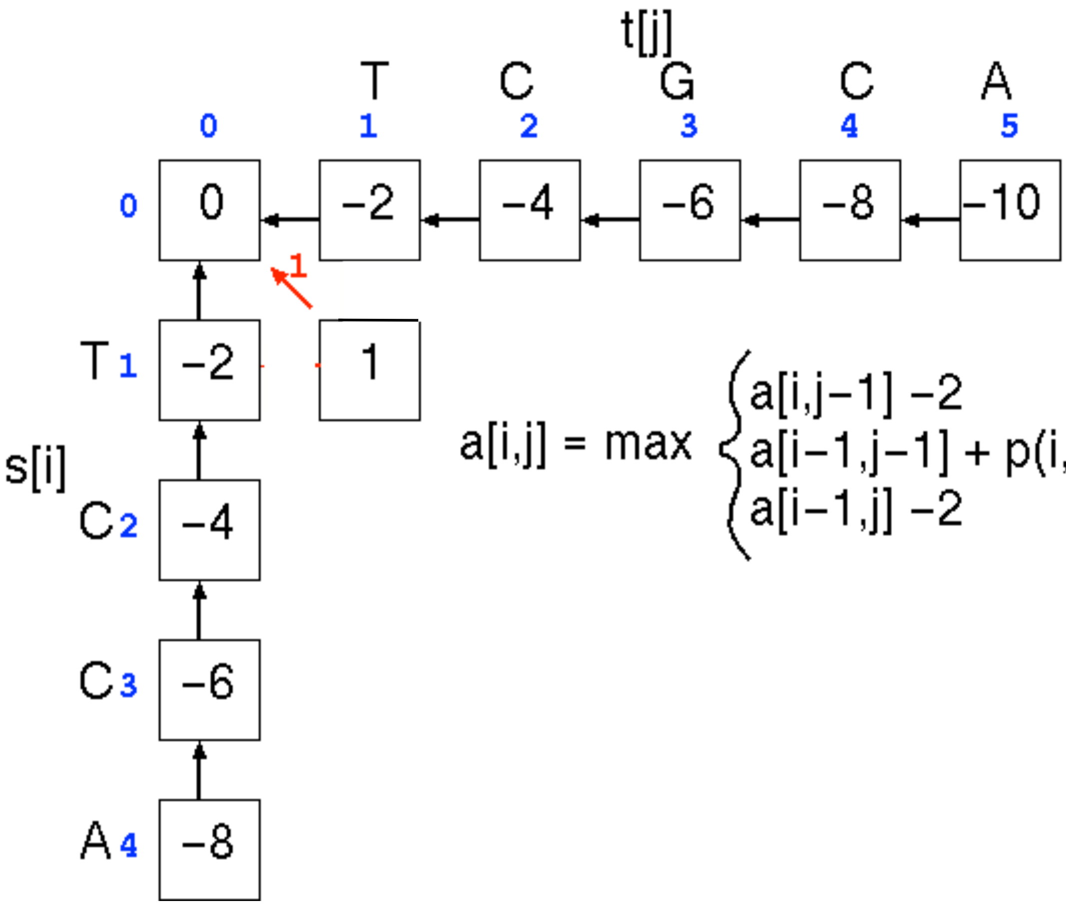
Gaps: -2

Dynamic programming: example



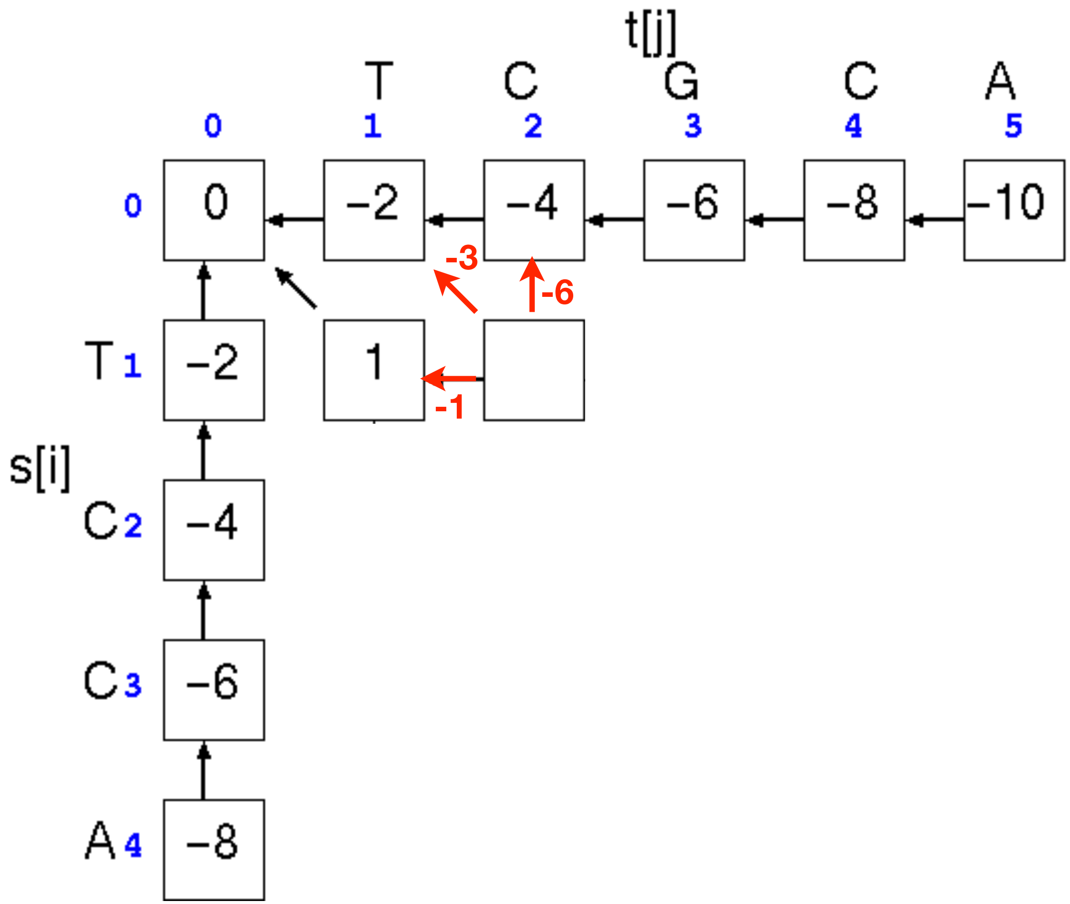
$$a[i,j] = \max \begin{cases} a[i,j-1] - 2 \\ a[i-1,j-1] + p(i,j) \\ a[i-1,j] - 2 \end{cases}$$

Dynamic programming: example

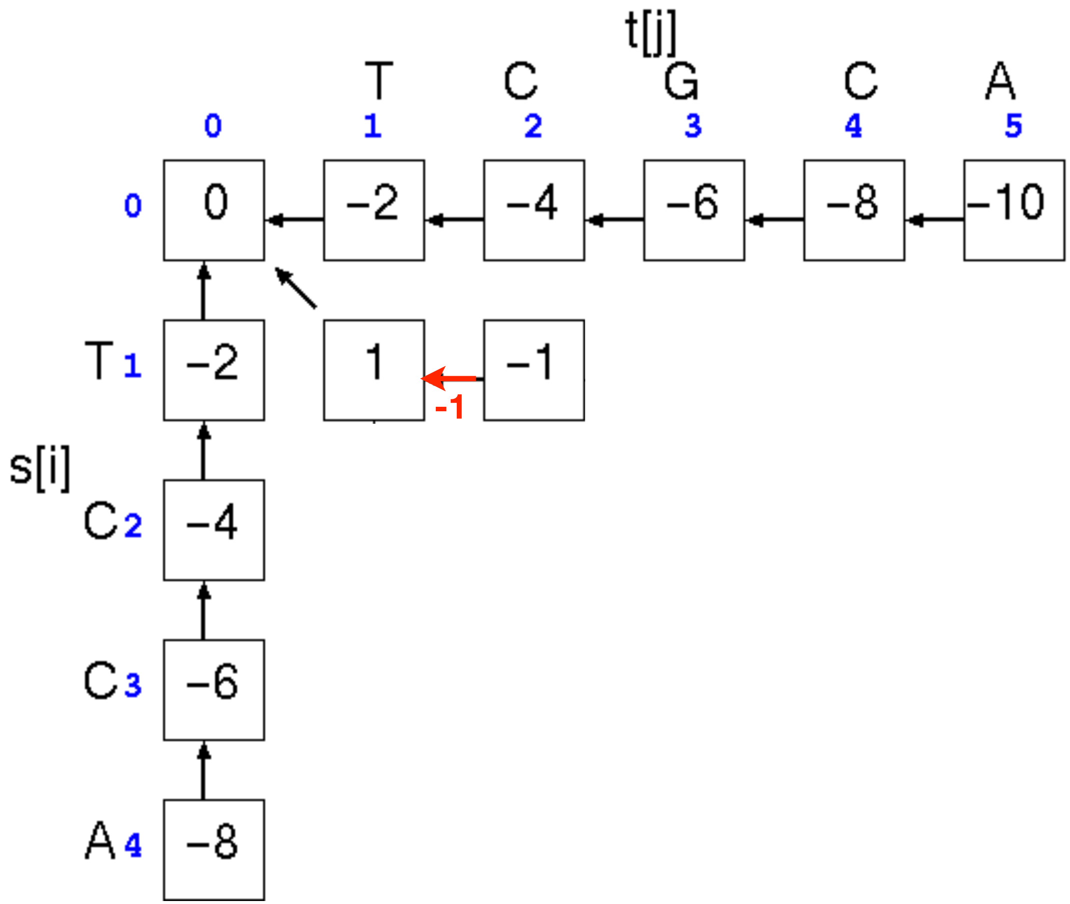


$$a[i,j] = \max \begin{cases} a[i,j-1] - 2 \\ a[i-1,j-1] + p(i,j) \\ a[i-1,j] - 2 \end{cases}$$

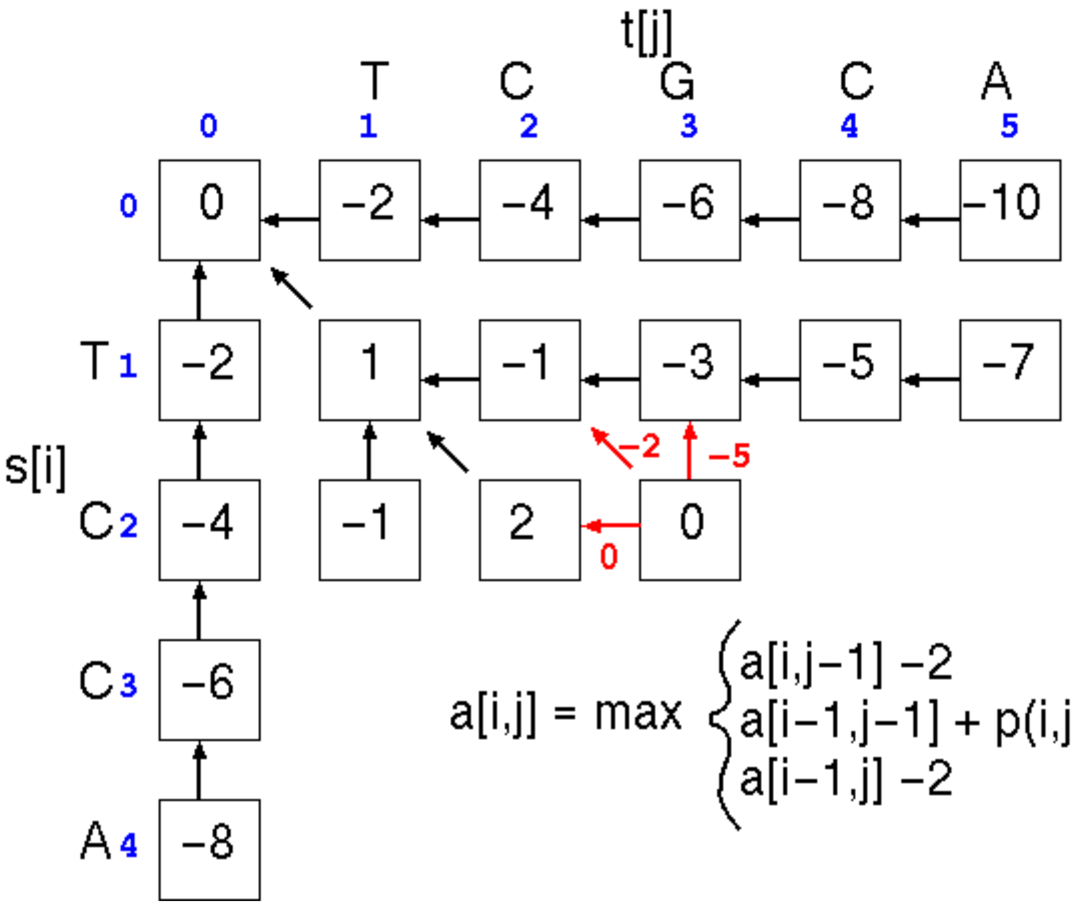
Dynamic programming: example



Dynamic programming: example

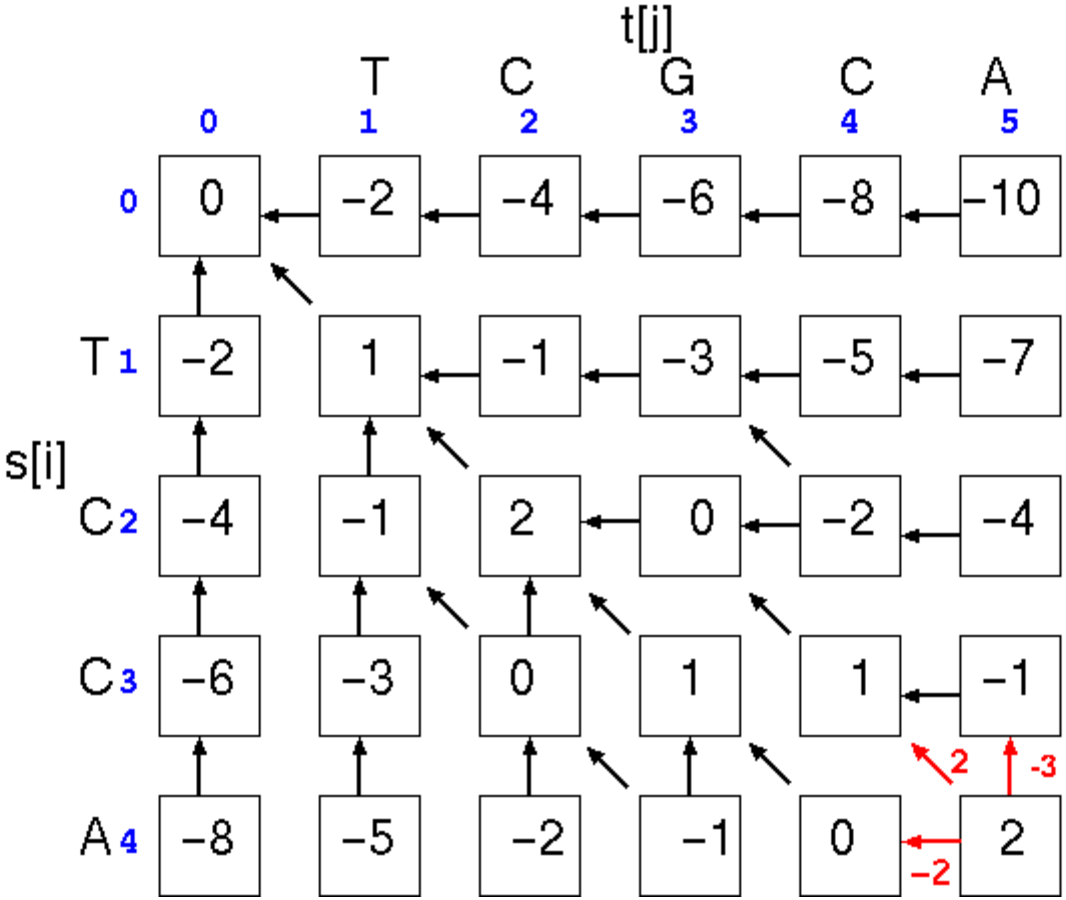


Dynamic programming: example

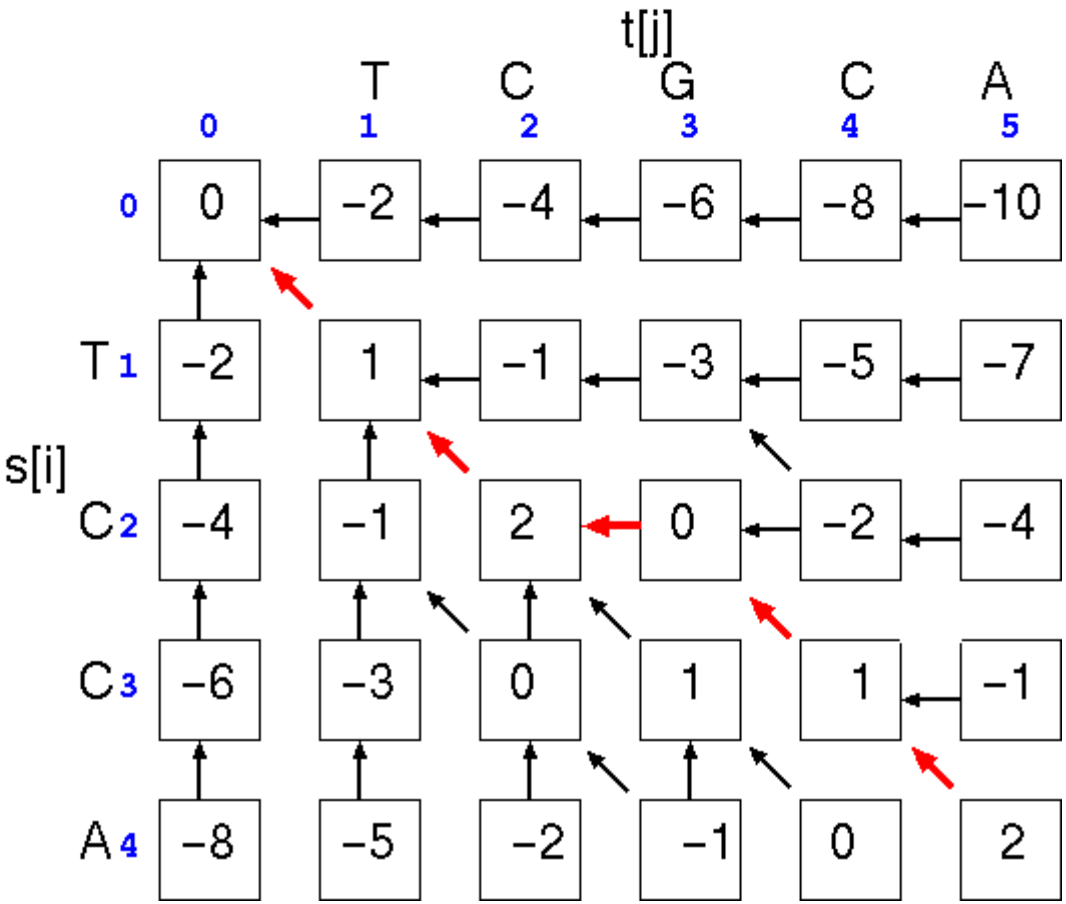


$$a[i,j] = \max \begin{cases} a[i,j-1] - 2 \\ a[i-1,j-1] + p(i,j) \\ a[i-1,j] - 2 \end{cases}$$

Dynamic programming: example



Dynamic programming: example



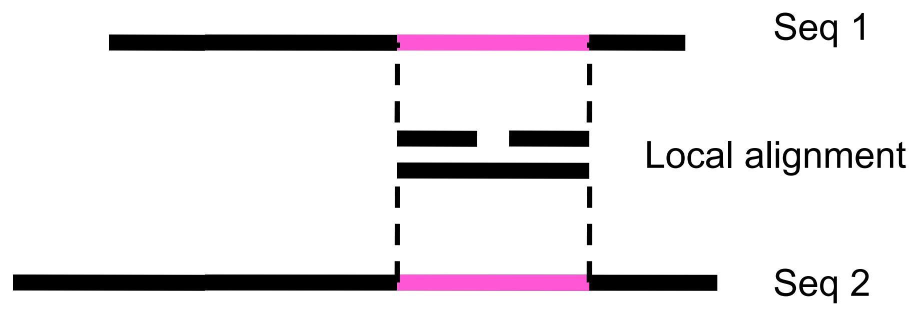

```
T C G C A
: :   : :
T C - C A
```

1+1−2+1+1 = 2

Global versus local alignments

Global alignment: align full length of both sequences.
(The “Needleman-Wunsch” algorithm).

Local alignment: find best partial alignment of two sequences
(the “Smith-Waterman” algorithm).



Local alignment overview

- The recursive formula is changed by adding a fourth possibility: zero. This means local alignment scores are never negative.

$$\text{score}(x,y) = \max \begin{cases} \text{score}(x,y-1) - \text{gap-penalty} \\ \text{score}(x-1,y-1) + \text{substitution-score}(x,y) \\ \text{score}(x-1,y) - \text{gap-penalty} \\ 0 \end{cases}$$

- Trace-back is started at the highest value rather than in lower right corner
- Trace-back is stopped as soon as a zero is encountered

Local alignment: example

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	5	0	**5**	0	0	0	0	0
W	0	0	0	0	2	0	**20**	**12**	4	0	0
H	0	10	2	0	0	0	12	18	**22**	14	6
E	0	2	16	8	0	0	4	10	18	**28**	20
A	0	0	8	21	13	5	0	4	10	20	27
E	0	0	6	13	18	12	4	0	4	16	26

```
AWGHE
AW-HE
```

Substitution matrices and sequence similarity

- Substitution matrices come as series of matrices calculated for different degrees of sequence similarity (different evolutionary distances).

- ”Hard” matrices are designed for similar sequences
  - Hard matrices a designated by high numbers in the BLOSUM series (e.g., BLOSUM80)
  - Hard matrices yield short, highly conserved alignments

- ”Soft” matrices are designed for less similar sequences
  - Soft matrices have low BLOSUM values (45)
  - Soft matrices yield longer, less well conserved alignments

Alignments: things to keep in mind

“Optimal alignment” means “having the highest possible score, given substitution matrix and set of gap penalties”.

This is NOT necessarily the biologically most meaningful alignment.

Specifically, the underlying assumptions are often wrong: substitutions are not equally frequent at all positions, affine gap penalties do not model insertion/deletion well, etc.

Pairwise alignment programs always produce an alignment - even when it does not make sense to align sequences.